WS21.5 Newborn screening for cystic fibrosis – Polish four years' experience with *CFTR* sequencing strategy

K. Wertheim-Tysarowska[1], A. Sobczyńska-Tomaszewska[1,2], M. Ołtarzewski[3], K. Czerska[1,2], D. Sands[3], J. Walkowiak[4,5], J. Bal[3], T. Mazurczak[3]. *[1]Institute of Mother and Child, Department of Medical Genetics, Warsaw, Poland; [2]Health Care Center Genomed, Warsaw, Poland; [3]Institute of Mother and Child, Warsaw, Poland; [4]Poznan University of Medical Sciences, Ist Chair of Pediatrics, Department of Gastroenterology & Metabolism, Poznan, Poland; [5]Poznan University of Life Sciences, Department of Dietetics, Poznan, Poland*

Objective: Newborn screening for cystic fibrosis (NBS CF) in Poland is a public health policy program started in September 2006. Summary from four years' experience is presented in this study.
Patients and Methods: The IRT/DNA strategy was implemented. DNA analysis was performed using direct sequencing of selected *CFTR* regions. The group of 1,212,487 newborns were screened for cystic fibrosis during the programme.
Results: A total of 221 CF cases were identified during this period. In addition, four CF cases were reported to be omitted by NBS CF programme. Disease incidence in Poland based on the programme results was estimated as 1/4394 and carrier frequency as 1/33. The frequency of the p.Phe508delCTT was similar (62%) to population data previously reported. This strategy allowed to identified twenty-nine affected infants with rare *CFTR* genotypes. The frequency of some mutations (for example c.2052_2053insA, p.Lys710X) was assessed in Poland for the first time.
Conclusions: Sequencing assay seems to be accurate method for screening programme using blood spots in Polish population.

WS21.6 11 years of newborn screening (NBS): the experience in Tuscany, Italy

T. Repetto[1], L. Zavataro[1], G. Mergni[1], I. Fusco[1], N. Vignoli[1], R. Pasotto[1], E. Pelo[2], C. Centrone[2], F. Torricelli[2], C. Braggion[1]. *[1]Cystic Fibrosis Centre, Meyer Children's Hospital, Florence, Italy; [2]Genetic Lab, Careggi Hospital, Florence, Italy*

Background: NBS program was established in Tuscany in 1982. In 2011 DNA mutation analysis was introduced to improve accuracy, taking into account costs and genetic background in our region.
Aims: To assess the performance of the NBS protocol in the period 2000–2010.
Methods: We reviewed the NBS data-base. Our three-stage protocol included:
1. an initial immunoreactive trypsin (IRT) measurement (99^{th} centile cut-off – Delfia™ and Autodelfia™);
2. meconium lactase dosage and a second IRT blood sample taken at 4 weeks of age;
3. the sweat test (ST).

Results: There were 347,815 babies screened in Tuscany in the period 2000–2010. Of these, 82 were diagnosed with CF and 9 (0.0026%) were known false negative cases. 12 infants were diagnosed by meconium ileus in this period and 10/12 were IRT-positive. Incidence of CF was 1 in 3,443 newborns. 12/92 (13%) had borderline ST at the diagnosis and 2 CFTR mutations. The median (IQR) age at the diagnosis was 46 (37, 57) days in the last 5-year period compared with 54 (48, 62) days in the previous years ($p < 0.05$). Considering the false negative cases, the median (IQR) age at diagnosis was 0.6 (0.3, 1.4) years; 3/9 had pancreatic insufficiency and respiratory symptoms, 5/9 had only salt loss as clinical features consistent with CF. Considering the 2006–2010 period, IRT-1 was positive in 0.91%; sensitivity, specificity, positive and negative predictive values were 92.98, 99.82, 13.80 and 99.99%, respectively.
Conclusions: Critical points in our program were a relatively late diagnosis age and the low positive predictive value. An IRT cut-off reassessment and the introduction of DNA analysis should improve the performance of our program.

WS21.7 Newborn screening for cystic fibrosis in Ontario, Canada: the first three years

S.A. Zelenietz[1,2], J. Milburn[1,2], J.L. Marcadier[1,2], M. Theriault[1,2], O.Y. Aldirbashi[1,2,3], M.T. Geraghty[2,3], P.K. Chakraborty[1,2,3]. *[1]Newborn Screening Ontario, Ottawa, Canada; [2]Children's Hospital of Eastern Ontario, Ottawa, Canada; [3]University of Ottawa, Ottawa, Canada*

Objective: To present the first three year's experience of NBS for Cystic Fibrosis (CF) in Ontario.
Methods: Dried blood spot specimens from infants were assayed for immunoreactive trypsinogen (IRT) via radio-immunoassay. Infants with IRT >96 centile had *CFTR* mutation analysis using the TM Biosciences Tag-It *CFTR* 39+3 mutation kit. Newborn Screening Ontario (NSO) created a categorical system to stratify screen positive results by risk: Category A: high IRT and two *CFTR* mutations, high risk (~100%); Category B: high IRT and one *CFTR* mutation, moderate risk (~2.5%); and Category C: elevated IRT above the 99.9 centile and no *CFTR* mutations, low risk (~1%). All infants with a screen positive result were referred for follow-up diagnostic testing at a regional NBS treatment centre.
Approximately 428,762 infants have been screened for CF; of those, 1257 were screen positive. NSO has identified 77 confirmed cases of CF. Eight hundred and seventy-one infants with a Category B screen positive have been confirmed to be unaffected carriers of CF. A subset of infants with borderline sweat chloride results ($n = 24$) are being followed to investigate the possibility of late onset *CFTR*-related disease. Follow-up data is pending on 96 infants.
Conclusions: After three years of screening, the screen positive rate for CF in Ontario is 0.29%. The positive predictive value of the test varies by screen positive result: 100% for Category A, 4.49% for Category B, and 1.29% for Category C. Infants with CF identified through NBS will be followed prospectively to determine if early detection improves the clinical course of disease.

WS21.8 Assessment of the UK CF newborn screening (NBS) programme; a protocol that uses a high cut-off for the first IRT measurement

P. Barton[1], P.S. McNamara[2], S. Bennett[1], E. Hanmer[1], K.W. Southern[3]. *[1]Alder Hey Children's Hospital Foundation Trust, Liverpool, United Kingdom; [2]University of Liverpool, Liverpool, United Kingdom; [3]University of Liverpool, Women's and Children's Health, Liverpool, United Kingdom*

Background: There is significant variation across the world with respect to the cut-off for the first IRT value (IRT-1) that triggers referral for further testing. In the UK, infants with an IRT-1 value above the 99.5^{th} centile are referred, whilst other countries have adopted lower cut-offs (as low as the 95^{th} centile). Taking the NBS blood spot sample after day 5 of life (as per the UK protocol) may result in a better differentiation between CF and non-CF, as IRT values in non-CF infants have been shown to fall in the first week of life.
Objective: To examine the performance of the UK protocol since implementation of NBS in February 2007.
Methods: We recorded
1. IRT-1 for all confirmed CF cases
2. IRT-1 for approximately 8000 consecutive infants in 2011 with a "CF not suspected" result and
3. IRT-1 for infants recognised as carriers.

Samples were excluded if they were repeats, contaminated, taken before D5 of life or after week 8. Results were from the NBS laboratory in Liverpool (screens approx 28,000 infants per year).
Results: For 75 infants with a confirmed diagnosis of CF, mean IRT-1 was 164.6 (SD, 82.4, lowest value 66.3). For 25 infants with a carrier result, mean IRT-1 was 81.5 (16.1). For 8052 randomly selected infants with a "CF not suspected" NBS result, the mean IRT-1 was 22.0 (10.5). There have been no false negatives reported in this group.
Conclusions: The UK protocol appears to be performing well with a relatively high IRT-1 cut-off. There is some overlap between "CF confirmed" and "CF not suspected" IRT-1 values but not significant. It appears valid to employ a cut-off of 99.5th centile when the IRT-1 sample is obtained on day 5 of life.